PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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19. Aug. 2005 BU
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Frist: PF

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	18.08.2005
Applicant's or agent's file reference: AX02A02/P-WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/006698	21.06.2004	20.06.2003

Applicant
AXIOGENESIS AG

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	de Haas, B Tel. +31 70 340-4738

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
AX02A02/P-WO		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/006698	21.06.2004	20.06.2003

International Patent Classification (IPC) or national classification and IPC
C12N5/06, C12N5/10, A61K35/34, C12Q1/02, A61P9/04

Applicant
AXIOGENESIS AG

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 13 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28.04.2005	18.08.2005

Name and mailing address of the international preliminary examining authority:

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/006698

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-29, 31-51	as originally filed
30	received on 28.04.2005 with letter of 28.04.2005

Claims, Numbers

1-81	received on 28.04.2005 with letter of 28.04.2005

Drawings, Sheets

1-3, 5-9	as originally filed
4	received on 28.04.2005 with letter of 28.04.2005

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 76-79

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 76-79

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/006698

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-56, 59-75
	No:	Claims	57, 58, 80, 81
Inventive step (IS)	Yes:	Claims	1-56
	No:	Claims	57-75, 80, 81
Industrial applicability (IA)	Yes:	Claims	1-16, 21-49, 57-75, 80, 81
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the report

1.1 The amendments filed with the letter of 28 April 2005 are allowable under Article 34(2)(b) and/or Rule 91 PCT, as applicable.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

3.1 Claims 17-20 and 50-56 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

3.2 No opinion will be formulated with respect to the reach-through claims 76-79 and those parts of claims 80 and 81 which refer to claims 76-79 because no International Search Report has been established for these claims (Rule 70.2(d) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

5.1 Reference is made to the following documents:

D1 WO 03/010303 A 6 February 2003

D1a Mummery C. *et al.*, *Circulation* 6 June 2003, 107(21), 2733-2740 (online 12 May 2003)

D2 WO 02/051987 A 4 July 2002

D3 US 5,733,727 A 31 March 1998

D4 Müller M. *et al.*, *FASEB Journal* December 2000, 14(15), 2540-2548

D5 Spielmann H. *et al.*, *Congenital Anomalies* December 2000, 40(Suppl.), S8-S18

D6 Kettenhofen R. *et al.*, *Naunyn-Schmiedeberg's Archives of Pharmacology* March 2002, 361 (Suppl. 1), R154, abstract 601

D7 Kolossov E. *et al.*, *Tissue Engineering* August 2003, 9(4), pages 853-854, abstract 230 Second Meeting of the European Tissue Engineering Society; Genoa, Italy, 3-6 Sept. 2003

5.2 Examples 1, 4 and 5 of D1 and the corresponding article D1a teach cardiomyocyte differentiation of embryonic stem cells (ES) by co-culture with visceral endoderm END-2 cells; examples 2 and 3 of D1 further describe the differentiation of ES into skeletal muscle cells and vascular endothelial cells by co-culturing ES with ectoderm or endoderm derived cells. However the endoderm or ectoderm cells used in the co-culture are not likely to integrate into growing tissue structures as they are mitotically inactivated.

5.3 D2 (examples) teaches the transfection of ES with resistance and reporter genes under the control of a cardiac-specific promoter and the selection of differentiated cardiomyocytes; applications of the cells in screening and therapy are foreseen (p. 37). Cardiomyocytes according to D2 and structures or implants containing these cardiomyocytes would normally neither be mixed with fibroblasts nor contain selection constructs for fibroblasts or endothelial cells. However, the vector constructs comprising resistance and reporter genes under the control of a cardiac-specific promoter would be useful for the methods of the invention if combined with further similar vectors featuring fibroblast- and/or endothelial promoters. The unclear ~~drafting of claim 57~~, which does not directly specify the technical features of the vector for which protection is sought (see point 8.2), may encompass a single vector of D2, to be then used in combination with further vectors, as well as one single vector containing all the constructs required by the method of claim 51 or a composition of vectors containing all the vectors required by the method of claim 51; under the first interpretation, claim 57 lacks novelty in view of D2. By way of reference to claim 57, it follows that the subject-matter of claims 57, 58, 80 and 81 is not novel (Article 33(2) PCT) and the subject-matter of claims 59-75 (since amended claim 62 now refers to claim 58) does not involve an inventive step over D2 (Article 33(3) PCT).

5.4 D3 (example 4) and D4 (see "Materials and Methods", p. 2541-2542) teach the differentiation of ES cells into cardiomyocytes and the selection of cardiomyocytes using a reporter gene under the control of a cardiac specific promoter; therapeutic applications are foreseen (D3, examples 1-3 and 5; D4, last paragraph, p. 2547), as well as screening assays (D4, last paragraph, p. 2547). D5 and D6 teach the use of ES transfected with a reporter gene under the control of a cardiac specific promoter and of cardiomyocytes derived from these ES in toxicity tests *in vitro*. As for D2 (see point 5.3 above), the vectors used in documents D3-D6 prejudice the novelty of claim 57, thus the subject-matter of claims 57, 58, 80 and 81 is not novel (Article 33(2) PCT) and the subject-matter of claims 59-75 (since amended claim 62 now refers to claim 58) does not involve an inventive step (Article 33(3) PCT).

5.5 The problem underlying the application is the provision of an alternative process for the derivation of cardiomyocytes and cardiac tissue from ES; the solution provided is a parallel selection process where ES cells are transfected with selectable markers under the control of promoters specific for cardiomyocytes and for fibroblasts and/or endothelial cells, allowing for the selection among the

differentiated cells of the desired cardiomyocytes together with cells supporting said cardiomyocytes. The closest prior art is represented equally by D1/D1a, which disclose the co-culture of ES with embryonic endothelial cells to support differentiation to cardiomyocytes, and by D2, which discloses a selection strategy for cardiomyocytes alone using selectable markers similar to those of the invention. None of the prior art documents suggests to derive simultaneously several cell types from ES and to allow these cell types to support each other and to give rise to cardiac tissue. The process of claims 21-42 is thus new and an inventive step can be acknowledged (Article 33(2,3) PCT).

5.6 The cells of claim 43 can be distinguished from similar products of the prior art, such as those described in D1/D1a and D2, in that, regardless of their type, the cells comprise selectable markers under the control of at least two regulatory sequences specific for different cell types. While these selectable marker constructs were known in the art (D2), and their presence does not provide the differentiated cells with any further property which would be useful with respect to therapy or screening assays, the prior art does not provide any incentive to transfect cells of one lineage with selection marker constructs for another lineage, as would be required to obtain cells identical to those of claim 43. Therefore, the cells of claim 43 meet the requirements of Article 33 PCT regardless of the process in which they are obtained.

5.7 It follows from points 5.5 and 5.6 above that the further products, methods and therapeutical uses of claims 1-20 and 44-56 also meet the requirements of Article 33 PCT. However, this Authority does not regard claims 1-17 and 21-50 as supported under Article 5 PCT for subject-matter other than cardiac tissue: See point 8.5 below.

5.8 For the assessment of the present claims 17-20 and 50-56 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims.

Re Item VI

Certain documents cited

6.1 D7 does not belong to the state of the art under Rule 64.1 PCT as the priority date of 20 June 2003 can be allowed for subject-matter derivable from the present examples 1-5.

Re Item VII

Certain defects in the international application (form, content)

7.1 With all respect due to Pr. McLuhan, this Authority questions the relevance of his observations about the media and their impact on the culture environment (Cf. p. 43, lines 6-7) for the understanding of the present application. For instance one may observe that a *medium*, in the sense given to this word in the field of technology to which to the present application belongs, does not appear to be able to *massage* the cardiac tissues of the examples.

Re Item VIII

Certain observations on the international application (clarity, support)

8.1 This Authority regards the number of claims, eighty-one in total of which no less than twenty-four are independent claims, as excessive and unreasonable in regard of the nature of the invention (Rule 6.1(a) PCT).

8.2 Claim 15 pertains to cells as defined in claims 1-14, while said claims 1-14 actually pertain to a method of modelling tissues; claim 57 pertains to vectors as defined in claims 51-56 while said claims 51-56 actually pertain to a method for improving cardiac function in a mammal; in both cases, the reader has to construe the technical features of the claimed products from the technical features of methods by which the products are produced (claims 1-14) or in which the products are used (claims 51-56) although the features of said methods impart properties onto the products only to a limited extent and, as a result, the matter for which protection is sought is not clearly defined (Article 6 PCT). While the applicant might have tried not to increase further the already excessive number of claims, the resulting intricacy in the set of claims between features of the methods and features of the product is not satisfactory with respect to clarity.

8.3 Claims 40-50, 59, 60, 62-75, 80 and 81 make use of dependency from multiple independent claims or refer to multiple independent claims, and of those, multiple dependent claims 45-50, 59, 60, 62-75, 80 and 81 make reference to multiple dependent claims in contravention of Rule 6.4 PCT. Whether this style of drafting is allowable under some national legislations is a moot point with respect to the PCT procedure (Cf. Article 35(2) PCT, first sentence)

8.4 Claim 61 does not meet the requirements of Articles 5 and 6 PCT in that the claimed apparatus is neither disclosed nor supported by the description: The description provides no actual guidance as

(e) subsequently co-transplanting said cardiomyocytes, fibroblasts and optionally endothelial cells or said tissue to at least a portion of the previously infarcted area of the heart tissue; and

(f) allowing said introduced cells or tissue to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

As mentioned before, said cardiomyocytes, fibroblasts and optionally endothelial cells are preferably derived from the same ES cell. However, cardiomyocytes, fibroblasts and optionally endothelial cells derived from different ES cells may be used as well. In those embodiments, said cardiac-specific regulatory sequence is preferably selected from promoters of αMHC, MLC2v, MLC1a, MLC2a and βMHC, said endothelium-specific regulatory sequence is preferably selected from promoters of Tie2, Tie1 and cadherin, and said fibroblast-specific regulatory sequence is preferably selected from promoters of collagen I; see supra. Similarly, said reporter for said cardiomyocytes, fibroblasts and optionally endothelial cells is independently preferably selected from the enhanced green fluorescent proteins ECFP (blue), EYFP (yellow) and hcRFP (red); see also Figure 3 and the examples. Said resistance gene and said reporter are preferably separated by an internal ribosomal entry site (IRES).

In another example, neuroepithelial cells are generated and used to augment or replace cells damaged by illness, autoimmune disorders, accidental damage, or genetic disorder. Mouse ES cells can be induced to differentiate in vitro with retinoic acid to form neuronal and glial precursors, positive for astrocyte (GFAP) or oligodendrocyte (O4) markers, then later into functional neurons (Fraichard et al., J. Cell Science 108 (1995), 3161-3188). Cells transplanted to adult brains were observed innervating the host striatum (Deacon et al., Exp. Neurology, 149 (1998), 28-41). Human and mouse EC cell lines can also differentiate into neurons. (Trojanowski et al., Exp. Neurology, 144 (1997), 92-97; Wojcik et al., Proc. Natl. Acad. Sci. USA, 90 (1993), 1305-1309). Transplantation of these neurons into rats subjected to cerebral ischemia promoted a degree of functional recovery (Borlongan et al., Exp. Neurology 149 (1998), 310-321). In accordance with the present invention, for this embodiment corresponding neuronal and glial specific promoters are used; see, e.g., Kawai et al., Biochim. Biophys. Acta 1625 (2003), 246-252, and Kugler et al., Gene Ther. 10 (2003), 337-347, for glial and neuronal specific promoters. Efficiency of embryoid body formation and hematopoietic development from embryonic stem cells in different culture systems is

Claims

1. Method of modeling and/or obtaining tissue or tissue-like structures comprising culturing an embryonic stem (ES) cell-derived first cell type in the presence of at least one embryonic second cell type; and allowing integration and alignment of said at least two cell types into tissue or tissue-like structures.

2. The method of claim 1, wherein the ES cell of said ES cell-derived first cell type comprises a selectable marker operably linked to a first cell type-specific regulatory sequence specific for said first cell type.

3. The method of claim 2, wherein said selectable marker confers resistance to puromycin.

4. The method of any one of claims 1 to 3, wherein said ES cell of said ES cell-derived first cell type comprises a reporter gene operably linked to a cell type-specific regulatory sequence specific for said first cell type.

5. The method of claim 4, wherein said cell type-specific regulatory sequence of the reporter gene is substantially the same as said first cell type-specific regulatory sequence of the marker gene.

6. The method of claim 5, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).

7. The method of any one of claims 4 to 6, wherein said marker gene and said reporter gene are contained one the same recombinant nucleic acid molecule.

8. The method of claim 7, wherein said marker gene and said reporter gene are contained on the same cistron.

9. The method of any one of claims 1 to 8, wherein said first cell type is selected from the group consisting of neuronal cells, glial cells, cardiomyocytes, glucose-responsive insulin-secreting pancreatic beta cells, hepatocytes, astrocytes, oligodendrocytes,

chondrocytes, osteoblasts, retinal pigment epithelial cells, fibroblasts, keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, vascular endothelial cells, testicular progenitors, smooth and skeletal muscle cells.

10. The method of any one of claims 1 to 9, wherein said first cell type are cardiomyocytes.

11. The method of claim 10, wherein said first cell type-specific regulatory sequence is atrial and/or ventricular specific.

12. The method of claim 10 or 11, wherein said at least one embryonic second cell type are fibroblasts or endothelial cells.

13. The method of any one of claims 1 to 12, further comprising culturing said at least two cell types in the presence of an embryonic or embryonic stem (ES) cell-derived third cell type.

14. The method of claim 13, wherein said third cell type are endothelial cells or fibroblasts.

15. A co-culture of cells as defined in any one of claims 1 to 14, comprising at least cells of said first and second cell type under conditions, wherein said cells are capable of integrating and alignment into tissue or tissue-like structures.

16. A tissue obtainable by the method of any one of claims 1 to 14.

17. A method of improving tissue repair and/organ function in a mammal comprising the steps of:

(a) introducing a cellular inoculum comprising a co-culture of cells of claim 15 in which differentiation has been initiated or tissue of claim 16 to at least a portion of the previously damaged area of the tissue; and

(b) allowing said introduced cellular inoculum to engraft in situ as viable cells or tissue situated within the previously damaged area of the tissue, wherein the engraftment results in improved tissue and/or organ function in said mammal.

18. A method for improving cardiac function in a mammal after a myocardial infarct, said method comprising the steps of:

(a) culturing undifferentiated mammalian embryonic stem (ES) cells comprising a resistance gene and a reporter gene under the control of the same cardiac-specific promoter in vitro in a culture medium containing the selective agent for the resistance gene under conditions allowing differentiation of said ES cells into cardiomyocytes;

(b) isolating said differentiated cardiomyocytes and/or eliminating non-differentiated cells, optionally along with cells differentiating towards irrelevant cell types from said cardiomyocytes in the course of differentiation;

(c) subsequently co-transplanting said cardiomyocytes with embryonic or ES cell-derived fibroblasts to at least a portion of the previously infarcted area of the heart tissue; and

(d) allowing said introduced cellular inoculum to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

19. The method of claim 18, wherein said resistance gene and said reporter gene are contained in a bicistronic vector and separated by an IRES.

20. The method of claim 19, wherein said resistance gene confers resistance to puromycin, said marker is EGFP and said promoter is the cardiac αMHC promoter.

21. Method of modeling and/or obtaining tissue or tissue-like structures comprising the following steps:

(a) transfecting one or more multi- or pluripotent cells with recombinant nucleic acid molecules comprising a first and a second cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said second cell type is different from said first cell type;

(b) culturing the cells under conditions allowing differentiation of the cells; and

(c) isolating cells of at least two differentiated cell types and/or eliminating non-differentiated cells, optionally along with cells differentiating towards

irrelevant cell types from cell types of interest that activate the selectable marker in the course of differentiation.

22. The method of claim 21, further comprising transfecting said one or more cells with recombinant nucleic acid molecules comprising at least one further cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said at least one further cell type is different from said first and second cell type.

23. The method of claim 21 or 22, wherein said cells are embryonic stem (ES) or embryonic germ (EG) cells.

24. The method of any one of claims 21 to 23, wherein said recombinant nucleic acid molecules are comprised in the same vector or different vectors.

25. The method of any of claims 21 to 24, wherein said cell type is selected from the group consisting of neuronal cells, glial cells, cardiomyocytes, glucose-responsive insulin-secreting pancreatic beta cells, hepatocytes, astrocytes, oligodendrocytes, chondrocytes, osteoblasts, retinal pigment epithelial cells, fibroblasts, keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, vascular endothelial cells, testicular progenitors, smooth and skeletal muscle cells.

26. The method of claim 7 or 8, wherein said promoter is selected from the group consisting of αMHC, MLC2V, cadherin, Tie-2 and collagen promoter.

27. The method of any one of claims 21 to 26, wherein said one or more recombinant nucleic acid molecules are transfected concomitantly or subsequently into said one or more cells.

28. The method of any one of claims 21 to 26, wherein at least two different cells or clones thereof are transfected and selected, wherein said at least two different cells or cell clones contain recombinant nucleic acid molecules with different cell type specific regulatory sequences.

29. The method of claim 28, wherein said at least two different cells or cell clones are mixed at the initial stage of differentiation in order to allow formation of cell aggregates.

30. The method of claim 29, wherein said cell aggregates are chimeric embryoid bodies (EBs).

31. The method of any one of claims 21 to 30, wherein one of said cells or cell clones thereof is transfected and selected, wherein said cell or cell clone contains recombinant nucleic acid molecules with at least two different cell type-specific regulatory sequences.

32. The method of any one of claims 21 to 31, wherein at least two of said selectable marker operably linked to said different cell type specific regulatory sequences are identical.

33. The method of any one of claims 21 to 32, wherein at least one of said selectable marker is operably linked to said different cell type-specific regulatory sequences confers resistance to puromycin, bleomycin, hygromycin, methothrexate, or neomycin.

34. The method of any one of claims 21 to 33, wherein one or more of said recombinant nucleic acid molecules further comprise a reporter operably linked to said cell type-specific sequence.

35. The method of claim 34, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).

36. The method of claim 35, wherein EYFP (yellow), ECFP (blue) and/or hcRFP (red) are operably linked to different cell type-specific sequences.

37. The method of any one of claims 34 to 36, wherein said selectable marker and said reporter are expressed from a bicistronic vector.

38. The method of claim 37, further comprising one or more internal ribosomal entry sites (IRES), wherein said IRES separates said selectable marker and said reporter.

39. The method of any one of claims 21 to 38, further comprising allowing self-assembly of the different cell types.

40. The method of any of claims 1 to 14 or 21 to 39, further comprising analysing the physiological and/or developmental status of the cells or cell aggregate.

41. The method of claim 40, wherein the status is analyzed by monitoring the differentiation of electrical activity of the cells on an array.

42. The method of claim 41, wherein said status is analyzed by recording the extracellular field potentials with a microelectrode array (MEA).

43. A cell or cells obtainable by the method of any one of claims 21 to 42, wherein said cell or cells are capable of differentiating into at least two cell types.

44. A cell aggregate of at least two different cell types obtainable by the method of any one of claims 21 to 42.

45. A tissue obtainable by the method of any one of claims 21 to 42 or comprising cells of claim 43 or a cell aggregate of claim 44.

46. An organ comprising cells of claim 43, a cell aggregate of claim 44 or tissue of claim 16 or 45.

47. An implant or transplant comprising cells of claim 43, a cell aggregate of claim 44, a tissue of claim 16 or 45, or an organ of claim 46.

48. A composition of matter comprising recombinant nucleic acid molecules as defined in any one of claims 21 to 42, comprising at least a first and a second cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said

second cell type is different from said first cell type, cells of claim 43, a cell aggregate of claim 44, or a tissue of claim 16 or 45.

49. Use of the method of any one of claims 1 to 14 or 21 to 42 for analyzing early steps of tissue formation during embryonic development or the influence of factors and compounds on this process.

50. A method of treatment of damaged tissue or organs in a subject comprising implanting or transplanting to the subject in need thereof cells of claim 43, a cell aggregate of claim 44, a tissue of claim 16 or 45 or an organ of claim 46.

51. A method for improving cardiac function in a mammal after a myocardial infarct, said method comprising the steps of:

(a) transfecting mammalian embryonic stem (ES) cells with a recombinant nucleic acid molecule comprising a resistance gene under the control of cardiac, and fibroblast and/or endothelium-specific regulatory sequences, and optionally comprising one or more reporters under the same specific regulatory sequences;

(b) culturing said ES cells in vitro in a culture medium containing the selective agent for the resistance gene under conditions allowing differentiation of said ES cells into cardiomyocytes, fibroblasts and/or endothelial cells;

(c) eliminating from said differentiated cardiomyocytes, fibroblasts and/or endothelial cells non-differentiated cells, optionally along with cells differentiating towards irrelevant cell types; optionally

(d) allowing aligning and integration of said differentiating cardiomyocytes, fibroblasts and/or endothelial cells into cardiac-like tissue;

(e) subsequently co-transplanting said cardiomyocytes and said fibroblasts and/or endothelial cells or said tissue to at least a portion of the previously infarcted area of the heart tissue; and

(f) allowing said introduced cells or tissue to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

52. The method of claim 51, wherein said cardiomyocytes, fibroblasts and/or endothelial cells are derived from the same ES cell.

53. The method of claim 51, wherein said cardiomyocytes, fibroblasts and/or endothelial cells are derived from different ES cells.

54. The method of any of claims 51 to 53, wherein said cardiac-specific regulatory sequence is selected from promoters of αMHC, MLC22v, MLC1a, MLC2a and βMHC, said fibroblast-specific regulatory sequence is selected from promoters of Tie2, Tie1 and cadherin, and said endothelium-specific regulatory sequence is selected from promoters of collagen I promoters.

55. The method of any of claims 51 to 54, wherein said reporter for said cardiomyocytes, fibroblasts and/or endothelial cells is independently selected from the enhanced green fluorescent proteins ECFP (blue), EYFP (yellow) and hcRFP (red).

56. The method of any of claims 51 to 55, wherein said resistance gene and said reporter are separated by an internal ribosomal entry site (IRES).

57. A vector or a composition of vectors comprising the recombinant nucleic acid molecules as defined in any one of claims 51 to 56.

58. A cell or a plurality of cells comprising the vector or the composition of vectors of claim 57.

59. An array comprising a solid support and attached thereto or suspended thereon cells of claim 43 or 58, a cell aggregate of claim 44, or a tissue of claim 16 or 45.

60. The array of claim 59, which is a microelectrode array (MEA).

61. An apparatus for analyzing the array of claim 59 or 60.

62. A method for obtaining and/or profiling a test substance capable of influencing cell development and/or tissue structure formation comprising the steps:

(a) contacting a test sample comprising cells of claim 43 or 58, a cell aggregate of claim 44, a tissue of claim 16 or 45, an organ of claim 46 or an array of claim 59 or 60 with a test substance; and

(b) determining a phenotypic response in said test sample compared to a control sample, wherein a change in the phenotypic response in said test sample compared to the control sample is an indication that said test substance has an effect on cell development and/or tissue structure formation.

63. The method of claim 62, wherein said test sample is contacted with said test substance prior to, during or after said cell or cell aggregate passed through the method of any one of claims 1 to 14 or 21 to 42.

64. The method of claim 62 or 63, wherein said contacting step further includes contacting said test sample with at least one second test substance in the presence of said first test substance.

65. The method of any one of claims 62 to 64, wherein preferably in a first screen said test substance is comprised in and subjected as a collection of test substances.

66. The method of claim 65, wherein said collection of test substances has a diversity of about 10^3 to about 10^5.

67. The method of claim 66, wherein the diversity of said collection of test substances is successively reduced.

68. The method of any one of claims 61 to 67, which is performed on an array as defined in claim 59 or 60.

69. The method of any one of claims 61 to 68, wherein the phenotypic response comprises electrophysiological properties during the ongoing differentiation process.

70. The method of any one of claims 1 to 14, 21 to 42 or 62 to 69, wherein said one or more cells are genetically engineered to (over)express or inhibit the expression of a target gene.

71. The method of any one of claims 1 to 14, 21 to 42 or 62 to 70, wherein a compound known to activate or inhibit differentiation process and/or tissue structure formation is added to the culture medium.

72. The method of any one of claims 1 to 14, 21 to 42 or 62 to 71, wherein said one or more cells or tissue are contained in a container.

73. The method of any one of claims 1 to 14, 21 to 42 or 62 to 72, comprising taking 3 or more measurements, optionally at different positions within the container.

74 The method of any one of claims 72 or 73, wherein said container is a well in a microtiter plate.

75. The method of claim 74, wherein said microtiter plate is a 24-, 96-, 384- or 1586- well plate.

76. A method of manufacturing a drug comprising the steps of any one of claims 62 to 75.

77. A method of manufacturing an agent which supports wound healing and/or healing of damaged tissue comprising the steps of any one of claims 62 to 76.

78. The method of claim 76 or 77, further comprising modifying said substance to alter, eliminate and/or derivatize a portion thereof suspected causing toxicity, increasing bioavailability, solubility and/or half-life.

79. The method of any one of claims 76 to 78, further comprising mixing the substance isolated or modified with a pharmaceutically acceptable carrier.

80. A kit or composition useful for conducting a method of any one of claims 1 to 14, 21 to 42, 50 to 56 or 62 to 79, containing the vector or the composition of vectors of claim 57, a multi- or pluripotent cell, and optionally culture medium, recombinant nucleic acid molecules, or standard compounds.

81. Use of cells of claim 43 or 58, a cell aggregate of claim 44, a tissue of claim 16 or 45 or an organ of claim 46, the implant or transplant of claim 47, the vector or the composition of vectors of claim 57, the composition of claim 48, an array of claim 59 or 60 or an apparatus of claim 61 in drug discovery or pharmacokinetic or pharmacological profiling.

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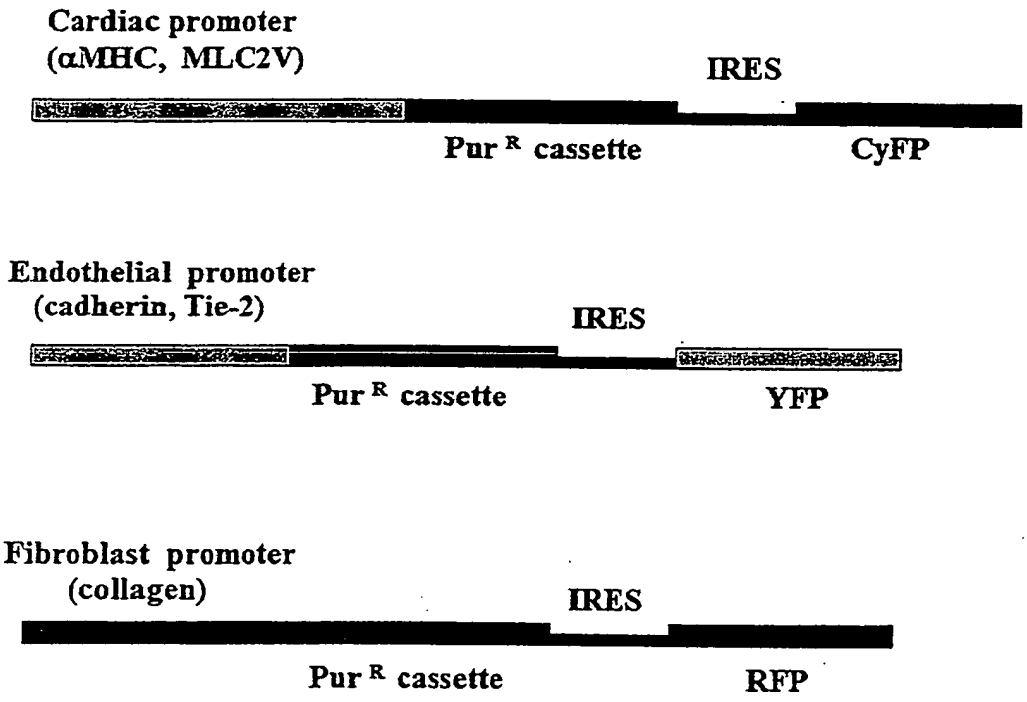

Figure 3